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CD40 on salivary gland epithelial cells: high constitutive expression by cultured cells from Sjögren's syndrome patients indicating their intrinsic activation

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SUMMARY

CD40 has been identified in an expanding list of haematopoietic and non-haematopoietic cells and has received an increased interest based on its role in a variety of cell-mediated responses and its potential to participate in the pathogenesis of chronic inflammatory disorders. Sjögren's syndrome (SS) is an autoimmune exocrinopathy, which is characterized by chronic lymphocytic infiltration of exocrine glands and aberrant activation of epithelial tissues. We studied the expression of CD40 protein in cultured non-neoplastic salivary gland epithelial cell (SGEC) lines as well as in minor SG biopsies obtained from 17 SS patients and 12 controls. Immunocytochemical and flow cytometric analyses had revealed the occurrence of constitutively expressed CD40 molecules on the surface of long-term cultured SGEC lines, which could be further induced by interferon-gamma (IFN-γ) and IL-1β cytokines, but not tumour necrosis factor-alpha (TNF-α), IL-4, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF) or IFN-α. Triggering of SGEC through CD40 enhanced the surface expression of the adhesion molecule intercellular adhesion molecule-1 (ICAM-1)/CD54, but not MHC class I and class II (HLA-DR) molecules. Spontaneous CD40 expression was significantly higher in SGEC lines derived from SS patients, compared with controls ($P < 0{\cdot}001$), which is suggestive of their intrinsically activated status. In SG biopsies, CD40 was constitutively expressed by lymphocytes, ductal epithelial cells and endothelial cells but not by other glandular cell types, such as acinar cells, myoepithelial cells and fibroblasts. In addition, CD40L staining was also detected in 30–50% of the infiltrating lymphocytes in the biopsies of SS patients. Our findings indicate the immunoregulatory potential of SGEC and lend further support to a model of intrinsic activation in salivary epithelia in SS, whereby these cells actively participate in the induction and maintenance of lymphocytic infiltrates of patients.

Keywords Sjögren's syndrome CD40 salivary glands epithelial cells

INTRODUCTION

Sjögren's syndrome (SS) is a chronic autoimmune disorder characterized by dysfunction and destruction of the salivary, lacrimal and other exocrine glands [1]. The glandular lesions are associated with lymphocytic infiltrates consisting of $CD4^+$ T cells that express various markers of activation [2]. During recent years, several lines of evidence have indicated that glandular epithelial cells in SS lesions are also aberrantly activated and may play an active role in the induction and perpetuation of the inflammatory processes [2]. We have previously proposed the term 'autoimmune epithelitis' to describe the involvement of epithelial tissue in the immune process of SS [3]. Recently, we demonstrated that long-term cultured non-neoplastic salivary gland epithelial cell (SGEC) lines derived from SS patients manifest increased constitutive surface expression of various immunoregulatory protein molecules, including costimulatory, adhesion and apoptosis-related proteins [4–6]. These findings strongly suggest the immunological function of SGEC and particularly the operation of intrinsic activation processes in the glandular epithelia of SS patients.

CD40 protein, originally identified as a B-cell antigen, drives the activation, proliferation and differentiation of B cells and rescues them from apoptosis [7–9]. Recently, constitutive or cytokine-induced expression of CD40 has been identified in a variety of neoplastic and non-neoplastic cells, which involve both lymphoid and non-lymphoid cells [10]. The latter include an expanding list of cell types such as epithelial cells [11–15], endothelial cells [16,17], muscle cells [18] and fibroblasts [19]. Signalling through CD40 has been shown to result in the induction of various immunological responses, including the expression of costimulatory and adhesion molecules and the production of inflammatory cytokines [20–22]. In this context, the CD40 pathway has been thought to participate in the pathogenesis of

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chronic inflammatory disorders and to represent a potential target for therapeutic immunological intervention [23,24].

In the present study, we sought to investigate the expression of CD40 protein by glandular epithelial cells and to address its implication in the chronic lymphoepithelial inflammatory lesions of SS. For this purpose, we examined the expression of CD40 molecules in cultured non-neoplastic SGEC lines, as well as in labial minor salivary gland biopsies obtained from SS patients and controls. SGEC were found to express considerable amounts of surface CD40 and this expression could be further up-regulated by interferon-gamma (IFN-γ) and IL-1β. Triggering of SGEC via CD40 was found to lead to the up-regulation of the surface expression of the adhesion molecule intercellular adhesion molecule-1 (ICAM-1/CD54). Long-term cultured SGEC lines from SS patients displayed significantly higher constitutive surface CD40 expression than controls, which further supports the hypothesis of intrinsic activation of these epithelial cells. This most certainly attests to the ability of SGEC to enhance the immune responses and underlines the importance of epithelial cells in the pathogenesis of SS lesions.

PATIENTS AND METHODS

Reagents and cell lines

Monoclonal antibodies (MoAbs) to human CD40 (clones EA-5 and BE-1), CD3, CD4 and CD8 were purchased from Ancell Corp. (Bayport MN, USA). A third antibody to human CD40 (clone 5C3), as well as the MoAb to human CD11a were from PharMingen (San Diego, CA). MoAbs to human CD40-ligand (CD40L, CD154), ICAM-1 (CD54), HLA-ABC, HLA-DR and to human cytokeratins 8 and 18 were purchased from Becton Dickinson (Mountain View, CA). MoAbs to human epithelial membrane antigen (EMA), CD20 and dendritic reticulum cell (DRC) were obtained from Dako (Glostrup, Denmark). Human recombinant epidermal growth factor (rEGF) was from Sigma (St Louis, MO). Human rIFN-γ(Imukin) was from Boehringer Ingelheim (Germany), human recombinant tumour necrosis factor-alpha (rTNF-α), rIL-4**,** rIL-6 and recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF) were from Endogen (Boston, MA) and rIL-1β was from R&D Systems (Minneapolis MN, USA). Human rIFN-α was from Schering-Plough (Kenilworth NJ, USA). The Epstein–Barr virus (EBV)-transformed B-cell line JY and Ramos lymphoma B cells (kindly provided by G. Thyphronitis, University of Athens, Greece) were cultured in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS), 5×10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Human umbilical vein endothelial cells (HUVEC) were grown in M199 medium (BRL, Grand Island, NY, USA) supplemented with 20% FBS, endothelial cell growth factor (50 μg/ml; Sigma), heparin (2 U/ml), penicillin (100 U/ml) and streptomycin (100 μg/ml). Peripheral blood B cells were isolated from healthy donors, as previously described [25]. Untransfected and hCD40L-transfected human kidney epithelial cell line (HEK, kindly provided by P. Sideras, AstraZeneka, Sweden) were grown in DMEM (GIBCO) supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin.

Minor salivary gland biopsies

Minor SG biopsies were obtained with informed consent from 29 individuals (27 women and two men) undergoing diagnostic evaluation for sicca symptoms indicative of SS [2]. SS patients in this study were diagnosed on the basis of the European SS classification criteria [26]. The control group consisted of 12 patients who did not fulfil the above criteria and had no histopathological evidence of SS. None of the patients had evidence of lymphoma, essential mixed cryoglobulinaemia or infection by hepatitis B virus, hepatitis C virus or HIV-1. Biopsy specimens were processed for tissue immunohistochemical analysis or for the development of epithelial cell cultures. For immunohistochemical analysis, biopsy specimens were paraffin-embedded and 4-μm sections of labial minor SG biopsy specimens were mounted on aminoalkylsilane (Sigma)-coated glass slides. The slides were dewaxed in xylol and a series of graded alcohol solutions before bringing in water.

Culture of non-neoplastic SGEC lines

Primary SGEC cultures were established from minor SG biopsies (one glandular lobule per patient) by explant outgrowth technique as previously described [4]. The serum-containing epithelial cell medium (SEM) and the serum-free keratinocyte basal medium (KBM; Clonetics, Walkersville, MD) were applied for the propagation of SGEC prepared as described before [4,27]. SGEC cultures were fed every 5 days with fresh culture medium and routinely subcultured at 70–80% confluence in appropriate bovine collagen type I-coated culture vessels. The epithelial origin of cultured SGEC lines was routinely verified by morphology as well as uniform and consistent staining with MoAbs to epithelial membrane antigen and the various cytokeratins, and absence of surface markers indicative for lymphoid cells (CD3, CD20, DRC) (data not shown). Published evidence from similarly established SGEC cultures is suggestive of their ductal epithelial origin [28]. No significant differences in morphology, overall survival or proliferation rates were observed between SGEC lines established from SS patients and controls. For immunocytochemical studies, SGEC lines (at second to fourth passage) were cultured onto eight- or 16-well multichamber slides (Nunc, Naperville IL, USA) for 5 days, at a density of $1{\cdot}5\times10^4$ or $0{\cdot}75\times10^4$ cells/well, respectively. Slides were washed in PBS, fixed in methanol/acetone (1 : 1) for 5 min at –20°C and processed for immunocytochemical staining. To study the regulation of CD40 expression by various cytokines, subconfluent (70–80% confluence) cultures of SGEC were treated with the following cytokines: IFN-γ, TNF-α, IFN-α, IL-4, IL-6 and GM-CSF (at 50, 100, 200 and 500 U/ml) and IL-1β (at 5 and 10 ng/ml), as defined previously [4]. The cytokines were applied for 24 h, 48 h and 72 h and the cells were collected by trypsinization, and then washed with PBS for flow cytometric analysis.

Immunohistochemistry and immunocytochemistry

Paraffin-embedded tissue specimens and multichamber slides with cultured SGEC lines from both SS patients and controls were analysed by immunohistochemistry or immunocytochemistry, respectively. An indirect avidin–biotin immunoperoxidase technique was employed in this study, as previously described [4]. Negative control stainings were performed by replacing primary antibodies (used at 1 : 10 to 1 : 25 dilution) with irrelevant isotype-matched antibodies. Normal horse serum (1·5%; Sigma) was used to block the non-specific binding, endogenous peroxidase activity was inhibited in 3% H_2O_2/in MetOH and the staining was developed using diaminobenzidine tetrahydrochloride (Dako). All sections were counterstained with haematoxylin. The staining

intensity (SI) of cultured cells was scored on an arbitrary scale from 0 to 3+ (0, absent; 1+, weak; 2+, definite; 3+, strong staining) by three independent observers.

Flow cytometric analysis

The analysis of the expression of cell surface CD40 protein was performed using FITC-conjugated MoAbs or isotype-matched controls (Becton Dickinson) in a standard staining procedure. Briefly, 5×10^4 cells were incubated with each antibody on ice for 30 min, washed with PBS containing 2·5% FBS and 0·3% NaN_3 and analysed with a FACSCalibur flow cytometer and CellQuest software (Becton Dickinson), with appropriate forward and side scatter adjustments for epithelial cells. Mean fluorescence intensity (MFI) values obtained by staining with specific MoAbs were corrected by the subtraction of background values (isotype-matched control MoAb).

Functional assessment of epithelial CD40 expression

To evaluate the function of surface CD40 protein molecules expressed by cultured SGEC, epithelial cell lines were cultured in the presence of fixed hCD40L-transfected HEK cells (HEK-CD40L) or untransfected controls and surface protein expression of ICAM-1, HLA-ABC and HLA-DR molecules was assessed. The efficiency and reproducibility of the ligation procedure applied were evaluated in preliminary experiments using the high CD40-expressing Ramos B cells, where triggering via surface CD40 was found to result in four-fold up-regulation of ICAM-1 expression (data not shown). In brief, HEK cells were trypsinized, fixed with 1% paraformaldehyde in PBS for 10 min and subsequently washed six times with PBS. In preliminary experiments, this fixation procedure was found to preserve the functional binding of CD40L to CD40-expressing B cells, while precluding HEK attachment to the culture vessels and any possible interference with the subsequent phenotypic analyses of SGEC. Fixed cells were reconstituted in KBM medium and applied on SGEC cultures for 24 h in a target-to-effector cell ratio ranging from 1:1 to 1:10. Subsequently, culture supernatants containing the HEK cells were removed and the expression of ICAM-1, HLA-ABC and HLA-DR by SGEC was assessed by flow cytometry, as above.

Statistical analysis

Where appropriate, comparison of data presented as the mean ± s.d. was performed using unpaired two-tailed Student's *t*-test and χ^2 test with Yate's correction, as well as by the non-parametric Wilcoxon tests for paired and unpaired measurements and Spearman rank correlation coefficient test.

RESULTS

Constitutive expression of CD40 molecules by cultured SGEC

Most cultured non-neoplastic SGEC lines were found to manifest considerable expression of surface CD40 molecules, as was indicated by immunocytochemistry and flow cytometry (Fig. 1). In preliminary experiments, the application of three different MoAbs to CD40 (clones EA-5, BE-1 and 5C3) had revealed comparable CD40 staining patterns between these MoAbs in a CD40-expressing EBV-transformed B cell line (JY) as well as in several long-term cultured non-neoplastic SGEC lines (data not shown). By immunocytochemistry, cultured SGEC presented CD40 expression as a diffuse surface staining pattern, which varied between the different cell lines (Fig. 1b). In each culture, the density of CD40 molecules was notably increased among a subpopulation of small, round proliferating cells. By flow cytometry, CD40 expression by SGEC manifested a bimodal pattern of

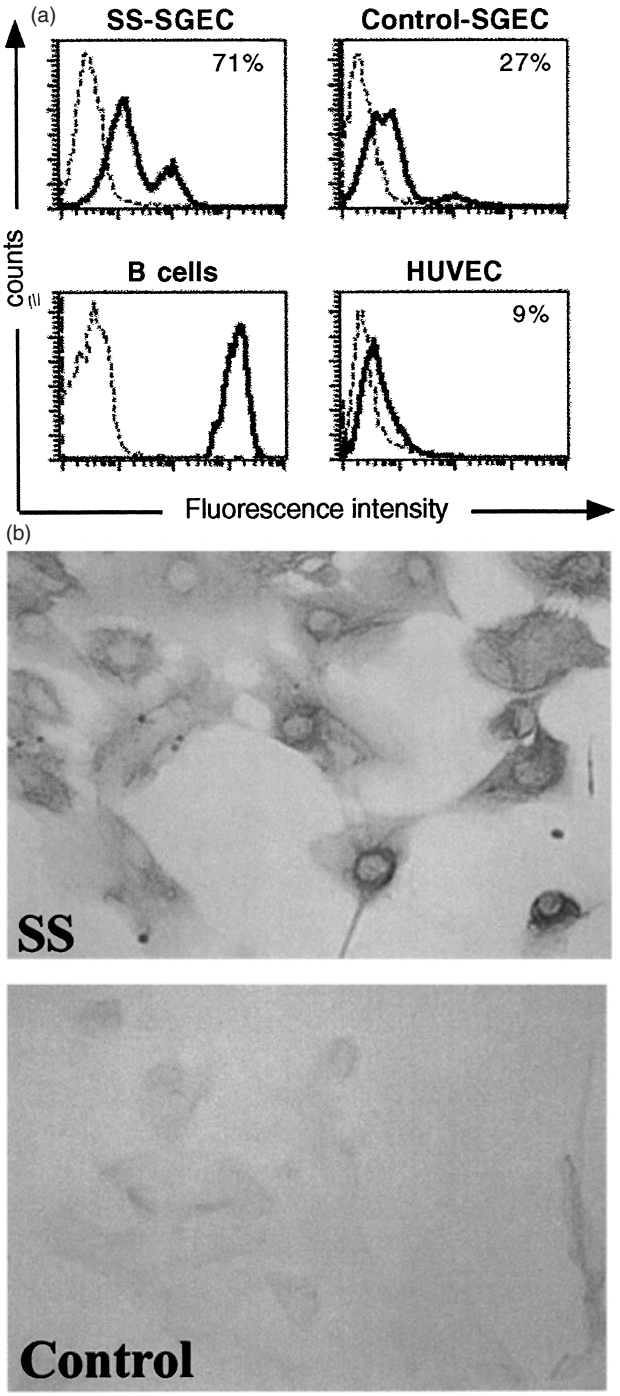

Fig. 1. Representative analyses of constitutive surface CD40 expression by salivary gland epithelial cell (SGEC) lines from a Sjögren's syndrome (SS) patient and a control individual, as indicated by flow cytometry (a) and immunocytochemistry (b). For comparison, CD40 expression by peripheral B cells and human umbilical vein endothelial cells (HUVEC) is also shown. Dotted histograms represent fluorescence staining levels obtained by the isotype-matched negative control MoAb.

staining consisting of two discrete subpopulations of low and high CD40-expressing epithelial cells, respectively (MFI of peaks: 9·3 ± 3·7 and 119 ± 56, respectively, $P < 0{\cdot}01$; Fig. 1a). The latter SGEC population ranged from 6% to 41% of total cells (median 11·5%) and consisted of small cells with low granularity, as indicated by analysis of the reciprocal forward and side scatter values (data not shown). CD40 expression by epithelial cells was found essentially unchanged through the long-term cultivation of established cell lines, as illustrated by successive immunocytochemical analyses at different passages (up to fifth), as well as after storage in liquid nitrogen (data not shown). Constitutive CD40 expression was significantly lower compared with that of the peripheral blood B cells, but stronger than that of human endothelial cells (HUVEC; Fig. 1a).

The analysis of immunocytochemical assays disclosed significantly higher constitutive CD40 expression on SGEC lines derived from SS patients (mean SI score 2·1 ± 0·6) compared with control cell lines, which presented minimal or no expression (mean SI score 0·6 ± 0·5, $P < 0{\cdot}001$; Table 1). This was also confirmed by flow cytometry, whereby SGEC obtained from SS patients manifested higher surface CD40 levels (MFI 103·1 ± 39·3) than controls (MFI 57·2 ± 14·2, $P < 0{\cdot}05$; Figs 1 and 2). There was no correlation between the level of CD40 expression and the focus score of SG biopsies.

Functional assessment of epithelial CD40 expression

The ligation of CD40 molecules expressed on SGEC by human CD40L-expressing HEK cells was found consistently to result in marginal up-regulation of ICAM-1 expression (Fig. 3), but not of HLA-ABC or HLA-DR (data not shown). The co-cultivation of SGEC with HEK-CD40L (but not with untransfected controls) led to substantial up-regulation of ICAM-1 expression (>25% induction of MFI values) in four out of five SGEC lines tested (three derived from SS patients and two derived from controls) (Fig. 3). No difference was observed in the responses between the high CD40 and low CD40-expressing cell lines derived from SS patients and control patients, respectively (Fig. 3).

Modulation of CD40 expression by cytokines

Certain proinflammatory and regulatory cytokines such as IFN-γ, TNF-α and IL-1β have been previously shown capable of modulating CD40 expression in non-lymphoid cells [11–14,16,19,29], and also to be produced in the lesions of SS patients [2]. Thus, to assess the influence of these and several other cytokines on CD40 expression by cultured non-neoplastic SGEC, low constitutive CD40-expressing cell lines derived from controls were selected and utilized. As for other membrane proteins [4–6], IFN-γ was found to up-regulate significantly surface CD40 ($P < 0{\cdot}001$; Fig. 4). CD40 up-regulation apparently occurred in all cultured

Table 1. Immunocytochemical analysis of CD40 expression in cultured salivary gland epithelial cell (SGEC) lines from Sjögren's syndrome (SS) patients and controls.

	CD40 staining intensity score			
	0	1+	2+	3+
SGEC lines (no. tested)	(No. of positive cell lines)			
Controls ($n = 5$)	2	3	–	–
SS ($n = 9$)	–	1	6	2

Results are expressed as staining intensity score on an arbitrary scale from 0 to 3+ (0, absent; 1+, weak; 2+, definite; 3+, strong staining).

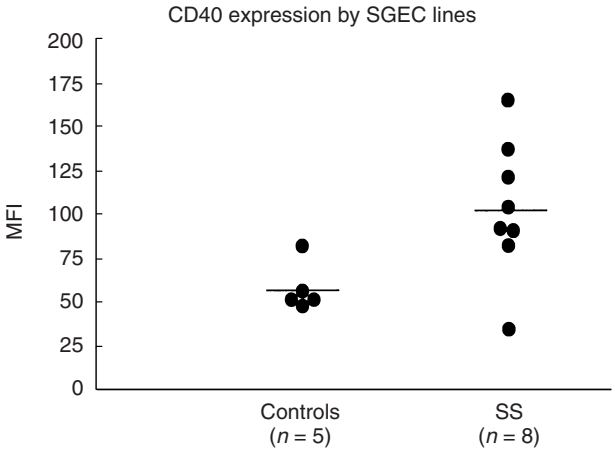

Fig. 2. Constitutive surface expression of CD40 protein by salivary gland epithelial cell (SGEC) lines derived from Sjögren's syndrome (SS) patients or controls, as assessed by flow cytometry and expressed as mean fluorescence intensity (MFI). Lines represent the median values.

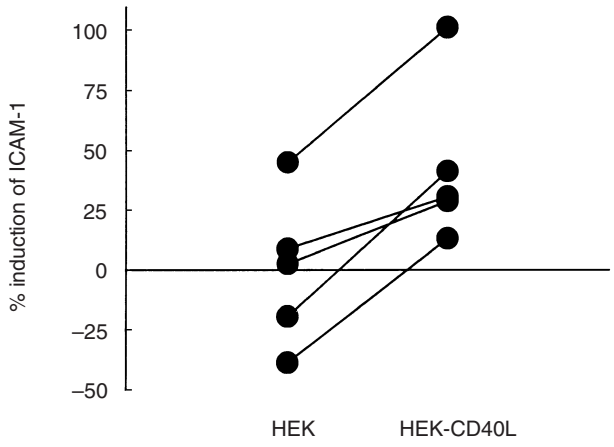

Fig. 3. Functional assessment of surface CD40 proteins expressed by cultured human non-neoplastic salivary gland epithelial cells (SGEC), as judged by flow cytometric analysis of surface intercellular adhesion molecule-1 (ICAM-1)/CD54 on cells subjected to ligation of CD40 or sham treatment. Data represent the percentage modulation of ICAM-1 expression in five SGEC lines co-cultured with untransfected HEK cells (HEK) or CD40L-transfected HEK cells (HEK-CD40L), as compared with ICAM-1 expression of cells treated with medium only (baseline). The mean (±s.d.) net percentage of ICAM-1 induction (i.e. the mean differences observed between the percentage of ICAM-1 induction obtained after treatment with HEK-CD40L and that obtained after treatment with untransfected HEK) was 43·3 ± 18·1 (median 52·1%). No evidence of differential response was noted between the high constitutive CD40-expressing cell lines (three lines derived from Sjögren's syndrome patients; net percentage induction values 60·5, 52·1 and 21·5) and the low constitutive CD40-expressing cell lines (two lines derived from controls; net percentage induction values 56·3 and 26·2).

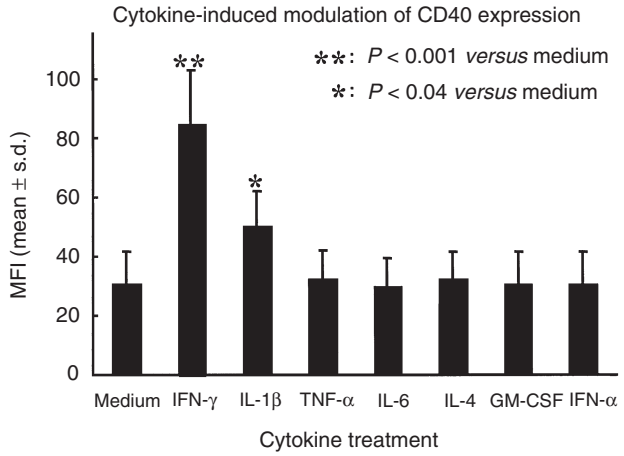

Fig. 4. Influence of various cytokines on surface CD40 protein expression by salivary gland epithelial cells (SGEC). Bars indicate mean (±s.d.) values of fluorescence intensities (mean fluorescence intensity (MFI)) observed following treatment of parallel SGEC cultures with medium only (medium) or with each of the cytokines indicated, for 24 h. Data are from five different SGEC lines, which were selected on the basis of low constitutive CD40 expression and were obtained from control individuals. Compared with treatment with medium only (median MFI value 30, values ranging from 13 to 45), significant up-regulation of CD40 was observed following incubation with IFN-γ (median MFI 88, ranging from 58 to 105, $P < 0{\cdot}001$) and IL-1β (median MFI 52, ranging from 34 to 65, $P < 0{\cdot}04$), but not after treatment with IL-4 (median MFI 32, ranging from 19 to 44), granulocyte-macrophage colony-stimulating factor (GM-CSF) (median MFI 30, ranging from 16 to 45), IFN-α (median MFI 30, ranging from 16 to 45).

cells and reached maximal levels by 24 h of IFN-γ treatment (approximately three-fold induction of MFI). The induction was less impressive in high CD40-expressing cells obtained from SS patients, probably suggesting a saturation effect (data not shown). The exposure of SGEC lines to IL-1β led to marginal but consistent up-regulation of CD40 expression ($P < 0{\cdot}04$), whereas treatment with TNF-α, IL-6, IL-4, GM-CSF or IFN-α had no apparent effect (Fig. 4).

CD40 and CD40L expression in salivary gland biopsies

To determine the occurrence of CD40 and CD40L molecules in the inflammatory lesions of SS, we examined the expression of both molecules on salivary gland biopsies obtained from SS patients and control individuals. Immunohistochemical analysis revealed high expression of CD40 molecules by round inflammatory cells, which appeared to be B cells, as judged by positive CD20 staining in adjacent sections of patients (Fig. 5A). Ductal epithelial and endothelial cells were also a source of considerable CD40 staining, which presented overall comparable intensity in biopsies derived from SS patients and controls (Fig. 5A,B). No evidence of CD40 expression could be detected on acinar cells, myoepithelial cells or fibroblasts. Immunohistochemical analysis also revealed the expression of CD40L in the inflammatory lesions of SG biopsies derived from SS patients. CD40L staining was detected in 30–50% of the infiltrating mononuclear cells (Fig. 5), which were presumably T lymphocytes, as indicated by their CD3 expression in serial sections (data not shown).

DISCUSSION

In SS patients, the salivary gland epithelia are the target of the inflammatory processes [2]. *In situ* tissue studies have previously indicated the aberrant activation of SGEC, and several lines of evidence suggest that these cells may participate in the induction and/or the maintenance of the inflammation [2]. Therefore, study of the immunological properties of SGEC is important for the understanding of the pathogenic processes in SS that we have previously described as 'autoimmune epithelitis' [3]. Our present findings provide evidence, for the first time, of the expression of CD40 protein by non-neoplastic SGEC. Although CD40 has been previously detected in a variety of cell types, to our knowledge the expression of CD40 protein by salivary epithelium has not been previously investigated in general, and particularly in the context of SS. The expression of CD40–CD40L by infiltrating lymphocytes has been recently observed in SG biopsies from SS lesions; however, no information related to CD40 expression by glandular epithelial cells has been provided [30].

As mentioned above, the activation of the glandular epithelial cells is probably the hallmark of pathophysiologic processes in SS [2]. We have previously presented evidence that this activation is sustained *in vitro*, as illustrated by the increased constitutive expression of various immunoregulatory and apoptosis-related proteins by long-term cultivated non-neoplastic SGEC that are established from patients with SS [4–6]. The present study suggests that this also applies to the constitutive CD40 expression, a fact which lends further support to our notion that the epithelial cells of SS patients are intrinsically activated [2,4]. At present, the mechanisms that cause this activation remain unclear and possibly involve the action of soluble autocrine mediators (e.g. cytokines) and/or the activation of strict intracellular pathways [4]. Nevertheless, SGEC activation is associated with the induction of an immunoregulatory phenotype on these cells, which may participate in the initiation and/or the perpetuation of autoimmune reactions in the syndrome. Preliminary evidence from our laboratory had revealed that cultured SGEC derived from SS patients produce increased amounts of the proinflammatory cytokines IL-1β and TNF-α. These cytokines do not seem solely responsible for the over-expression of CD40 molecules. In fact, our data imply the operation of an autocrine mechanism that may involve the above-mentioned proinflammatory cytokines as well as other, yet unidentified, factor(s).

On the other hand, CD40 expression may be also up-regulated by cytokines that are produced in the inflammatory lesions of SS. IFN-γ, a cytokine produced by the infiltrating T cells in the lymphoepithelial lesions of SS, can up-regulate the expression of CD40 by SGEC, in a manner similar to several other types of cells [12–14,16,17,19]. Interestingly, the induction of CD40 by other cytokines appears to be largely dependent on tissue-specific mechanisms. Among IL-1β, TNF-α, IL-4 and GM-CSF, which have been previously shown to induce CD40 expression on various cell lines, only IL-1β marginally up-regulated the expression of CD40 on SGEC.

Immunohistochemical analysis in SG biopsies from SS patients and controls revealed that CD40 is constitutively expressed by ductal epithelial cells but not by other epithelial cell types, such as acinar and myoepithelial cells, or fibroblasts. Endothelial cells were also a noticeable source of CD40 expression, as also previously observed [16]. In addition, in agreement with a previous report [30], high expression of CD40L proteins

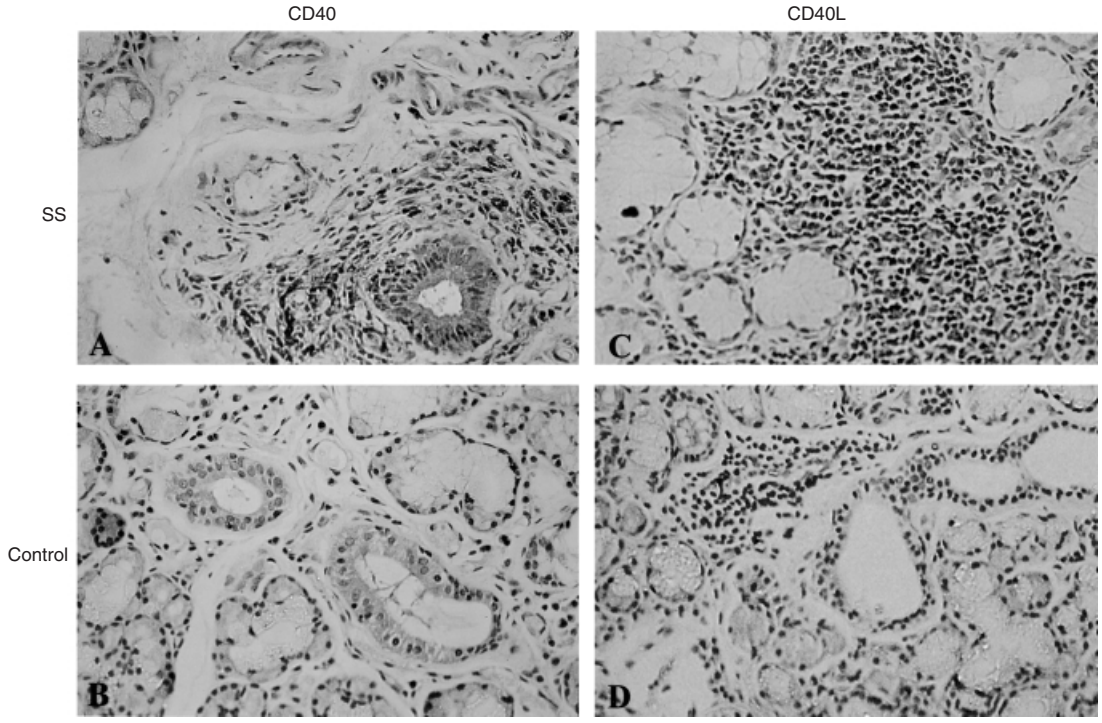

Fig. 5. Representative salivary gland biopsy specimens from patients with primary Sjögren's syndrome (SS) (A,C) and control (B,D) featuring CD40 (left) and CD40L (right) expression (×200).

was also identified on the infiltrating lymphocytes of SS lesions, a fact that is compatible with the activation phenotype of these inflammatory cells. In contrast to the detection of up-regulated surface CD40 expression on SGEC cell lines from SS patients, we could not definitely establish a similar difference in the *in situ* CD40 expression between SS patients and controls, probably owing to the inaccuracy of the immunohistochemical detection for quantitative estimations. Based on its well-established immunological function on various types of non-lymphoid cells [11,12,17,18,31–34], the expression of surface CD40 by SGEC in the face of CD40L-expressing infiltrating lymphocytes is conceivably of immunoregulatory importance. The interaction of epithelia and infiltrating T lymphocytes via CD40–CD40L probably leads to the generation of bi-directional activation signals to both cell types that can lead to the perpetuation of inflammatory reactions. We are currently investigating the involvement of such interactions in T-cell activation and proliferation and in the regulation of immunological activities of glandular epithelia. At present, our findings indicate that in a manner comparable to other types of non-haematopoietic cells [12,16,31], CD40 expression on SGEC is functional. The ligation of surface CD40 molecules was found to result in marginal but consistent up-regulation of ICAM-1 expression, which probably denotes a functional role of CD40 on SGEC in the intensification of immunological interactions between activated T lymphocytes and epithelia.

Apart from its function in immune responses, recent experimental evidence had indicated the importance of CD40 pathway for the regulation of growth and differentiation of epithelial tissues [29,35]. The high expression of CD40 by undifferentiated cells of normal skin specimens, such as the basal dermal keratinocytes, has been associated with the high proliferation rate of these cells [36]. In line with this, the occurrence of high CD40 levels in a subpopulation of cultured SGEC appears to be also associated with their active proliferation, as indicated by their small size and low granularity. In the same context, the preferential and high CD40 expression by ductal epithelium in the salivary glands probably reflects the presence of actively proliferating cells. It is of particular interest that the triggering of CD40 has been shown to inhibit the proliferation of keratinocytes and to result in the differentiation of cells [29,35]. In a similar fashion, signalling through CD40 has been shown to lead to growth inhibition in various malignant cell lines [37]. In this context, the triggering of salivary epithelia via CD40 by CD40L-expressing T cells in the inflammatory lesions of SS patients may play an adverse role in the tissue renewal and repair processes of epithelia.

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